IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Toshio Sone et al. Art Unit: 1644
Serial No.: 09/142,524 Examiner: DiBrino, M.
Filed: 01/04/99
Title: Peptide-based Immunotherapeutic Agent for Treating Allergic Diseases

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

I, Kohsuke Kino, declare that:

1. I am one of the inventors of the above-identified U.S. patent application.

2. I am making this Declaration to provide relevant facts in support of the patentability of the subject matter claimed in the patent application.

3. I have read and understood the outstanding Office Action mailed August 16, 2000.

4. I understand that the Examiner rejects Claims 1, 3-7, 10-13 and 17-26 under 35 U.S.C. §103(a) as being unpatentable over Rogers et al in view of secondary references and contends that, our arguments that Rogers et al fail to teach polypeptides which do not substantially react with IgE antibodies found in sera of allergic patients, whereas the peptides of the instant invention failed to substantially react with immune sera of tested individuals, are not persuasive because the statistical significance of the claimed invention with regard to "fail to substantially react" with IgE from allergic individuals cannot be assessed since no standard deviation values are given.

5. To demonstrate that the statistical significance of the claimed invention, I have analyzed the data shown in Example 7 and Fig. 8 of the instant specification statistically as described below.

6. The six multi-epitope peptides (C.A.#1 through C.A.#6) obtained in Example 6 were dissolved in 0.2 M acetate buffer solution (pH 4.5). The solution was dispensed in quantities of 0.1 ml/well

in a black plate (manufactured by Dainihon Pharmaceutical Co., Ltd.) then allowed to stand at 4°C overnight. After the antigen solution was removed, the wells were washed three times with a washing solution and the serum (4-fold dilution) from 21 patients with cedar pollinosis and 8 healthy subjects (29 subjects in total) were each added to separate wells. The system was then reacted at 37°C for 4 hours. After the sera were removed, the wells were washed three times with a washing solution then reacted with β-D-galactosidase-labeled anti-human IgE antibody (made by Pharmacia Inc.) at room temperature overnight. After washing three times with a washing solution, a substrate solution containing 0.1 mM 4-methylumbelliferyl-β-D-galactopyranoside/0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% NaN_3 and 0.1% BSA was added, and the solution was incubated at 37°C for 2 hours. A solution of 0.1 M glycine/NaOH (pH 10.3) was added to the wells to terminate the reaction. Fluorescent intensity was measured using a fluorophotometer (Labsystems). For positive control to each multi-epitope peptide, biotin-labeled rabbit anti-d epitope IgG and galactosidase-labeled streptavidin (made by Pierce Inc.) were reacted.

7. Figure 8 shows the results. The sera of 21 patients with cedar pollinosis (sample No. 1-21 in Fig. 8) exhibited a fluorescence intensity of 2105 to 45 to Cry j 1 extracted and purified from cedar pollen (544.7±522.7), while the sera of 8 healthy subjects (sample No. 22 to 29 in Fig. 8), 14 to 3 (average: 7.0±4.8). Aspin-Welch test revealed a significant difference with a significant level of 0.1% between the two groups. On the other hand, sera from 21 patients exhibited a fluorescent intensity of 3 to 5 to each of the six multi-epitope peptides (C.A.#1 through C.A.#6). In contrast, sera from 8 healthy subjects exhibited a fluorescent intensity of 3 to 4 to the six multi-epitope peptides. Thus, as is clear from Fig. 8, without statistically analyzing these data, it is obvious that there is no difference between sera of cedar pollinosis patients and healthy subjects in terms of reactivity of the multi-epitope peptides with sera. Likewise, there is no difference between a blank value obtained in the patient group (fluorescence intensity: 3 to 4) and that of the healthy subject group (fluorescence intensity: 3.0). Furthermore, fluorescent intensity values to the six multi-epitope peptides obtained in the two groups are comparable to blank values

obtained in the two groups.

8. These results indicate that the multi-epitope peptides of the present invention do not substantially react with cedar pollen allergen-specific IgE antibodies in sera of cedar pollinosis patients. Furthermore, the order of joining each T cell epitope peptide in the multi-epitope peptide of the present invention does not affect the reactivity of the multi-epitope peptide with cedar pollen allergen-specific IgE antibodies.

9. I further declare that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

January 5, 2001.

Date

Kohsuke Kino

Fig. 8

Reactivity of Peptide Compositions (#1 - #6) with Human IgE

Sample No. (sera)	Blank	Cedar pollen-extracted antigen	C.A.# 1	C.A.# 2	C.A.# 3	C.A.# 4	C.A.# 5	C.A.# 6
1	3	2105	5	4	3	4	4	4
2	3	1133	4	4	4	4	4	4
3	3	1126	3	3	3	4	4	3
4	3	1095	4	3	3	3	3	3
5	3	1047	3	3	3	3	3	3
6	3	1003	3	4	3	3	3	3
7	4	710	4	4	4	4	4	4
8	3	521	3	3	3	3	3	3
9	3	314	3	3	4	3	4	4
10	3	298	3	3	4	4	4	3
11	3	279	3	3	3	3	3	3
12	3	253	3	3	3	3	3	3
13	3	239	3	3	3	3	3	3
14	3	235	4	4	3	3	3	3
15	3	233	3	3	3	3	4	3
16	3	226	4	4	3	3	3	3
17	3	190	3	3	3	3	3	3
18	3	162	4	4	4	4	4	4
19	3	123	3	3	3	3	3	3
20	3	106	3	3	3	3	4	3
21	4	45	3	3	3	3	3	4
22	3	14	3	3	3	3	3	3
23	3	13	3	3	3	3	3	3
24	3	11	3	3	3	3	3	3
25	3	5	4	3	3	3	4	4
26	3	4	4	4	3	4	4	3
27	3	3	3	3	3	3	3	3
28	3	3	3	3	3	3	3	3
29	3	3	3	4	3	3	3	3
Rabbit anti-peptide IgG	112	230	3754	3829	3769	3716	3841	3798